Effects of Glucose Concentration on Biomass, Maximum Specific Growth Rate and Extracellular Enzyme Production by Three Species of Cutaneous Propionibacteria Grown in Continuous Culture

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Three cutaneous propionibacteria – *Propionibacterium acnes*, *P. avidum* and *P. granulosum* – were grown in chemostats using semi-synthetic medium with various concentrations of glucose. Biomass rose with increasing glucose concentration up to 0.3–0.4% (w/v) and then remained constant. *Propionibacterium granulosum* had both a low yield and low $\mu_{\max}$ when grown in the absence of glucose suggesting that this organism is essentially 'saccharolytic' in its nutrition. In contrast, *P. acnes* and *P. avidum* had higher growth yields than *P. granulosum* and showed their highest $\mu_{\max}$ values in the absence of glucose. Extracellular lipase, hyaluronidase (hyaluronate lyase) and acid phosphatase activities varied with different glucose concentrations, but in all cases were lowest at the highest glucose concentration tested (0.5% w/v).

INTRODUCTION

There are three described species of cutaneous propionibacteria – *Propionibacterium acnes*, *P. avidum* and *P. granulosum* (Johnson & Cummins, 1972). These three species produce a variety of extracellular enzymes including lipase (Hassing, 1971; Kellum *et al.*, 1970; Ingham *et al.*, 1981), hyaluronidase (hyaluronate lyase) (Puhvel & Reisner, 1972; Ingham *et al.*, 1979), protease, DNAase (Marples & McGinley, 1974) and acid phosphatase (Greenman *et al.*, 1979; Ingham *et al.*, 1980). These substances may be important in the initiation of inflammation in acne (Holland *et al.*, 1978).

It has been shown previously that changes in both oxygen tension and pH can affect the biomass and extracellular enzyme production of *P. acnes* grown in batch culture using complex medium (Holland *et al.*, 1978). Batch culture studies using synthetic medium have shown that the nature of the carbon/energy source can also affect the biomass and extracellular enzymes of the cutaneous propionibacteria (Holland *et al.*, 1979).

The aim of the present comparative investigation was to determine the effect of changing the concentration of glucose on the physiology of *P. acnes*, *P. avidum* and *P. granulosum* grown in chemostat cultures; extracellular enzyme production was of particular interest.

METHODS

Organisms and media. *Propionibacterium acnes* [laboratory strain P37], *P. avidum* [laboratory strain PF77(1)] and *P. granulosum* [laboratory strain PF208(11)] (note that this strain wrongly appears as *P. avidum* in Table 1 of Holland *et al.*, 1979) were isolated from patients attending the Dermatology Clinic at Leeds General Infirmary, and were typed according to the scheme of Marples & McGinley (1974). The strains were maintained at –196 °C under liquid nitrogen. The growth medium was that used by Eaves *et al.* (1979) without thiamin. Sterile glucose solution (10%, w/v) was added aseptically to the sterile bulk medium to give the final concentration required.

Continuous culture apparatus and conditions. Organisms were grown in a 1 litre culture vessel with control modules for temperature, pH, gas flow and stirrer rate (Series 500, L.H. Engineering, Stoke Poges, Bucks, U.K.). The growth conditions were those used by Eaves *et al.* (1979). The dilution rate was maintained at 0.012 h^{-1} for *P. granulatum*, at 0.040 h^{-1} for *P. acnes* and at 0.053 h^{-1} for *P. avidum*. These rates were chosen because of the different maximum specific growth rates of the three species. Samples were taken at regular intervals for microscopical examination and plating on RCM medium (Oxoid), which was incubated anaerobically in H_2/CO_2 (95:5, v/v) at 37°C for 5 d, and on 5% (w/v) heated horse blood agar (Oxoid), which was incubated aerobically for 2 d at 37°C , to test for purity.

Determination of biomass and maximum specific growth rates. Bacterial dry weight was used as a measure of the biomass. Cells were deposited by negative pressure filtration on to pre-dried and pre-weighed cellulose acetate filters (25 mm diam., $0.45\text{ }\mu\text{m}$ porosity; Oxoid). The filters were then dried for 30 min under an infrared lamp, cooled to room temperature and re-weighed. The bacterial dry weight was obtained from the difference in the weights.

The maximum specific growth rate (μ_{max}) was determined by the 'washout method' of Tempest (1970) by plotting the dry weight against time and calculating μ_{max} from the slope of the curve, making use of the equation: $\ln(N_1/N_0) = (\mu_{\text{max}} - D)(t_1 - t_0)$, where D is the dilution rate above μ_{max} , and N_0 and N_1 are bacterial concentrations (as measured by dry weight) at times t_0 and t_1 , respectively, during the washout phase of the culture. The dilution rate was raised to near μ_{max} 48 h before it was raised above μ_{max} . The values of D above μ_{max} were 0.30 , 0.28 and 0.29 h^{-1} for *P. acnes*, 0.34 , 0.36 and 0.32 h^{-1} for *P. avidum*, and 0.24 , 0.28 and 0.28 h^{-1} for *P. granulatum*, at glucose concentrations of 0 , 0.2 and 0.5% (w/v), respectively.

Extracellular enzymes. Lipase (EC 3.1.1.3), hyaluronidase (EC 4.2.99.1) and acid phosphatase (EC 3.1.3.2) activities were assayed by the methods used by Holland *et al.* (1979). All activities were expressed as $\mu\text{mol end-product h}^{-1} (\text{mg dry wt cells})^{-1}$.

Glucose. Glucose in the medium was assayed by the method of Huggett & Nixon (1957).

Phosphorus. Total phosphorus in the medium was determined by the method described in the Ministry of Agriculture, Fisheries and Food (1973) *Technical Bulletin 27*; phosphates were determined according to Chen *et al.* (1956).

Steady state. Biomass and extracellular enzyme activities were determined on samples taken from the chemostat during the particular conditions of nutrition that were selected. Only after a minimum of six culture volume changes had occurred between particular conditions was a steady-state condition considered to have been achieved.

RESULTS

Cell biomass and extracellular enzyme production were measured for each species of *Propionibacterium* grown at steady state, with the glucose concentration in the growth medium increased from 0 to 0.5% (w/v) in 0.1% (w/v) increments.

Biomass

The biomass increased until the glucose concentration reached 0.3% (w/v) for *P. avidum* and *P. granulatum* and 0.4% (w/v) for *P. acnes*. Biomass then remained constant or decreased slightly with further increases in the glucose concentration (Fig. 1). Under 'no glucose' conditions, *P. acnes* and *P. avidum* had approximately fourfold higher initial yields than did *P. granulatum*. At glucose concentrations between 0 and 0.3% (w/v) (Fig. 1) *P. avidum* and *P. granulatum* showed a greater increase in biomass (0.27 g l^{-1}) than did the *P. acnes* strain (0.11 g l^{-1}).

Extracellular enzyme production

Lipase activity of *P. acnes* increased with increasing glucose concentration up to 0.3% (w/v). At this point the lipase specific activity was twice that under 'no glucose' conditions. At higher glucose concentrations the activity decreased (Fig. 2). For *P. avidum*, the lipase activity remained almost unchanged up to 0.3% (w/v) glucose, was higher with 0.4% (w/v) glucose, but fell again when the concentration was increased to 0.5% (w/v). Lipase activity of *P. granulatum* was very high when the cells were grown without glucose. This activity decreased when the cells were grown on 0.1% (w/v) glucose, remained nearly constant up to

Fig. 1

Fig. 2

Fig. 1. Effect of glucose concentration on the biomass of *P. acnes* (○) ($D = 0.04 \text{ h}^{-1}$), *P. avidum* (●) ($D = 0.05 \text{ h}^{-1}$) and *P. granulosum* (□) ($D = 0.01 \text{ h}^{-1}$). Each point and bar indicates the mean $\pm$ standard deviation for nine separate samples.

Fig. 2. Effect of glucose concentration on the production of lipase by *P. acnes* (○), *P. avidum* (●) and *P. granulosum* (□). Lipase activities are expressed as μmol oleic acid released h^{-1} (mg dry wt cells)⁻¹. Other details as for Fig. 1.

Fig. 3

Fig. 4

Fig. 3. Effect of glucose concentration on the production of hyaluronidase by *P. acnes* (○) and *P. granulosum* (□). Hyaluronidase activities are expressed as μmol N-acetylglucosamine released h^{-1} (mg dry wt cells)⁻¹. Other details as for Fig. 1.

Fig. 4. Effect of glucose concentration on the production of acid phosphatase by *P. acnes* (○). Acid phosphatase activities are expressed as μmol p-nitrophenol released h^{-1} (mg dry wt cells)⁻¹. Other details as for Fig. 1.

0.3% (w/v) glucose, and finally decreased again at higher glucose concentrations to about a quarter of that in the absence of glucose.

Hyaluronidase was produced only by the *P. acnes* and *P. granulosum* strains (Fig. 3). The pattern of production for these two strains was similar. Specific activity was about 50% higher with 0.2% (w/v) glucose than without glucose. As the concentration was progressively increased above 0.2% (w/v), hyaluronidase activity steadily decreased.

Table 1. *Effect of glucose concentration on the maximum specific growth rate of P. acnes, P. avidum and P. granulorum*

Species	Glucose concn (%, w/v)	$\mu_{\max}$ (h ⁻¹)
<i>P. acnes</i>	0	0.16
	0.2	0.09
	0.5	0.09
<i>P. avidum</i>	0	0.18
	0.2	0.08
	0.5	0.10
<i>P. granulorum</i>	0	0.02
	0.2	0.06
	0.5	0.03

Extracellular acid phosphatase was detected only in the *P. acnes* strain (Fig. 4). The enzyme specific activity was the same with 0, 0.1 or 0.2% (w/v) glucose in the growth medium. With 0.3% (w/v) glucose and above, activity was much lower (about one-third of that in the absence of glucose).

Maximum specific growth rates

Values of $\mu_{\max}$ obtained by the 'washout method' are shown in Table 1. The *P. acnes* and *P. avidum* strains showed similar trends in that the highest values for $\mu_{\max}$ were obtained when they were grown without any glucose in the medium (0.16 and 0.18 h⁻¹, respectively). In contrast, *P. granulorum* showed its lowest value for $\mu_{\max}$ (0.02 h⁻¹) when grown under these conditions.

The values of $\mu_{\max}$ for *P. acnes* and *P. avidum* at 0.2 and 0.5% (w/v) glucose were similar (between 0.08 and 0.10 h⁻¹) and were approximately half that of the $\mu_{\max}$ in the 'no glucose' environment. In contrast, the $\mu_{\max}$ of *P. granulorum* was about three times higher in the presence of 0.2% (w/v) glucose (0.06 h⁻¹) than in its absence. With 0.5% (w/v) glucose, $\mu_{\max}$ was again low (though not as low as in the absence of glucose).

DISCUSSION

Ideally, experiments comparing specific growth rates, biomass and extracellular enzyme production should be performed with carbon-limiting and nitrogen-limiting conditions. However, as *P. acnes*, *P. avidum* and, to some extent, *P. granulorum* can utilize amino acids as carbon/energy as well as nitrogen sources, and as the nutritional requirements are complex (Ferguson & Cummins, 1978; Holland *et al.*, 1979), simple carbon-limiting conditions using glucose alone could not be obtained. Therefore, the experiments to determine $\mu_{\max}$ were conducted using the following three conditions: no glucose, 0.2% (w/v) glucose and excess glucose. Glucose at 0.2% (w/v) probably represents carbon/energy limitation, because the addition of more glucose resulted in a higher biomass and less than 1 µg glucose ml⁻¹ was detected in the cultures. Although the growth limitation in media with 0.5% (w/v) glucose could not be defined, phosphate was not limiting in these experiments.

Incremental yields of *P. acnes* with increasing glucose concentrations were smaller than those of *P. avidum* and *P. granulorum* indicating a more efficient use of glucose by the latter two species.

The *P. granulorum* strain showed differences to the *P. acnes* and *P. avidum* strains used in this study. Its $\mu_{\max}$ was lowest and it had a very low biomass when grown under 'no glucose' conditions. This result is in agreement with an earlier report (Holland *et al.*, 1979) that *P. granulorum* failed to grow in a synthetic medium containing a high concentration of arginine but grew in the same medium with a low arginine concentration; neither medium contained

glucose. At low arginine concentrations the biomass was similar to that obtained in the present study using 'no glucose' tryptone medium. However, with *P. acnes* and *P. avidum* the biomass was lower in the synthetic medium than in tryptone medium. This effect may be due to the differences in the culturing systems and the lack of peptides in the synthetic medium. The metabolism of *P. granulosum* is basically 'saccharolytic' and it is unable to utilize amino acids or small peptides efficiently as carbon/energy sources. It is possible that in the protein-rich skin environment *P. acnes* and *P. avidum* rely on proteinases (Marples & McGinley, 1974) to scavenge for amino acids and peptides as a carbon/energy source, whereas the non-proteolytic *P. granulosum* is dependent on other carbon/energy sources.

Under the conditions tested, *P. acnes* and *P. avidum* showed their highest $\mu_{\max}$ when grown under 'no glucose' conditions. Presumably a variety of metabolic pathways and permease transport systems are available to permit these organisms to utilize the different amino acids present in the medium. In the presence of glucose, the $\mu_{\max}$ of both *P. acnes* and *P. avidum* was >40% lower than under 'no glucose' conditions. This might be explained by glucose repression of the synthesis of enzymes important in obtaining carbon/energy from amino acids.

Of the three species tested, *P. avidum* seems the most nutritionally adaptable because it grew well in 'no glucose' conditions and responded to glucose addition with a greater increase in biomass than the other two species. However, on the skin *P. acnes* is the most commonly found species, whilst *P. avidum* is restricted in habitat (McGinley *et al.*, 1978). This would suggest that nutrient adaptability is not the major factor controlling the population of these species on human skin.

Extracellular enzymes may function to supply the organisms with carbon/energy sources *in vivo*. Lipase could provide the organisms with a source of glycerol, which they are known to be able to utilize (Holland *et al.*, 1979), from triglycerides found in sebum (Rebello & Hawk, 1978), although triglyceride would not be a very efficient substrate for providing the organisms with energy.

Lipase production by *P. granulosum* (Fig. 2) was highest when the cells were grown without glucose and this production was reduced by the presence of glucose (0.1%, w/v), suggesting repression of lipase synthesis. The effect of glucose on lipase production by *P. acnes* and *P. avidum* was opposite to that of *P. granulosum*, in that they showed increases in lipase activity with the addition of glucose. The control over lipase production in *P. acnes* and *P. avidum* in response to changes in glucose concentration seems to be weak, with maximum changes of twofold or less compared with a fourfold change for *P. granulosum*. The different response of *P. granulosum* might be explained by its greater dependence on fermentable substrates, and for this species greater control over lipase production would be an advantage. The other two species would benefit little from such control. It is notable that *P. granulosum* produced 20 times more lipase per unit biomass than either *P. acnes* or *P. avidum*.

Extracellular enzyme activities were lowest when glucose was at its highest concentration (0.5%, w/v). This trend might indicate that so-called catabolite repression (Magasanik, 1961) is occurring, whereby a freely available carbon/energy source represses the synthesis of enzymes now redundant since they are concerned with acquiring more carbon/energy. This type of repression also allows conservation of nitrogen needed for more important cellular functions in the, possibly, nitrogen-limited conditions. However, more drastic reduction in extracellular enzyme production by low concentrations of glucose would be expected if this was the control mechanism. Only with the acid phosphatase of *P. acnes* and the lipase of *P. granulosum* does this type of repression seem to occur and even then it is not complete.

This study suggests that physiological responses in *P. granulosum* differ from those of *P. acnes* and *P. avidum*, and *P. granulosum* may rely on different mechanisms to *P. acnes* and *P. avidum* to survive on the skin. Until more information is available on the micro-environment of the skin, the physiology of the bacteria living there will remain a matter of conjecture.

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